DATA EVALUATION RECORD

N,N-Dimethyldodecylamine oxide

Study Type: 84-2; Cell Transformation/*Salmonella typhimurium* Gene Mutation Assays

Work Assignment No. 3-27 (MRID 44434908)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Syrian hamster embryo cell transformation/*Salmonella typhimurium* Gene Mutation assays (870.5265; §84-2

DP BARCODE: D244775 SUBMISSION CODE:S538885
P.C. CODE: 000439

TEST MATERIAL (PURITY): N,N-Dimethyldodecylamine oxide (29.1% a.i.)

SYNONYMS: none

CITATION: Inoue, K., Sunakawa, T., and Takayama, S (1980) Studies of *In Vitro* Cell Transformation and Mutagenicity by Surfactants and Other Compounds. Tochigi Research Laboratories, Kao Soap Co. Ltd., 2606, Akabane, Ichikairmachi, Haga-gun, Tochigi and Department of Experimental Pathology, Cancer Institute, Kami-Ikebukuro, Toshima-ku, Tokyo, Japan. MRID 44434908. Published in Food and Cosmetics Toxicology, Vol. 18 pp. 289-296, 1980.

SPONSOR: The Proctor & Gamble Company, Cincinnati, OH

EXECUTIVE SUMMARY:

In a published study (MRID 44434908), N,N-dimethyldodecylamine oxide (29.1% a.i.) in dimethylsulfoxide or distilled water, was tested as one of a series of surfactants in three runs of an *in vitro* mammalian cell transformation assay using Syrian hamster embryo cells at concentrations of 0.1-20 µg/mL, and in a single microbial gene mutation assay using *Salmonella typhimurium* strains TA98 and TA100, with and without metabolic activation at concentrations of 10-200 µg/plate. In the cell transformation assay, cultures were exposed to the test compound for eight days prior to evaluation for transformed colonies. In the microbial gene mutation assay, the standard plate incorporation test with a preincubation step was performed; S9 homogenates for metabolic activation were made from polychlorinated biphenyl induced rat livers. No exogenous metabolic activation system was used in the cell transformation assay.

N,N-dimethyldodecylamine oxide was moderately toxic to Syrian hamster embryo cells at 20 µg/mL and was extremely toxic to *S. typhimurium* strains TA98 and TA100 at 200 µg/plate in the absence

of metabolic activation. N,N-dimethyldodecylamine oxide did not induce transformation in Syrian hamster embryo cells and was not mutagenic in *S. typhimurium* strains TA98 and TA100 with or without metabolic activation at any concentration. Findings with the positive controls confirmed the sensitivity of the microbial gene mutation assay to detect mutagenesis. However, a weak response with the positive control in the transformation assay reduced the sensitivity of the test system to detect transformation.

This study is **unacceptable according to FIFRA Test Guideline §84-2 (not upgradable)** and cannot be upgraded for the following reasons: (i) the positive control in the transformation assay did not produce the appropriate response, (ii) the microbial gene mutation assay employed only two instead of the recommended four tester strains of *S. typhimurium*, (iii) the highest dose level in the microbial gene mutation assay was well below the recommended limit dose and toxicity was not reported with metabolic activation at any dose level. In addition, the lot number of the test compound was not provided and the number of plates used at each dose level in the microbial gene mutation assay was not reported.

COMPLIANCE: A signed and dated Good Laboratory Practices Statement indicated that the submitter was not the original sponsor and did not conduct the study; therefore, it was unknown whether the study was conducted in accordance with 40 CFR §160. In addition, a signed and dated Statement Of No Confidentiality was provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: N,N-Dimethyldodecylamine oxide
   Description: Liquid
   Lot/Batch #: Not reported
   Purity: 29.1% a.i.
   Stability of compound: Not reported
   CAS #: Not provided
   Structure: Not provided
   Solvent used: Dimethylsulfoxide (DMSO) or distilled water
   Other comments: The transformation assay included a medium control. In the gene mutation assay, distilled water or DMSO served as solvent for the tested compounds. The study did not specify the solvent used with N,N-dimethyldodecylamine oxide.

2. Control Materials:

   a. Transformation assay

   Solvent/final concentration: DMSO/0.2%
   Positive: 3-methylcholanthrene (3-MC), 0.1, 0.5, and 1.0 µg/mL.

   b. Gene mutation assay

   Negative: Solvent served as negative control
   Solvent/final concentration: DMSO or distilled water/not reported

   Positive:
   Nonactivation:

4-Nitroquinoline 1-oxide	0.5 µg/plate	*S. typhimurium* TA98 and TA100
N-Methyl-N'-nitro-N-nitrosoguanidine	2 µg/plate	*S. typhimurium* TA100

Activation:

2-Acetylaminofluorine	50 µg/plate	*S. typhimurium* TA98 and TA100
Benzo[a]pyrene	5 µg/plate	*S. typhimurium* TA98 and TA100
N-nitrosodimethylamine	3000 µg/plate	*S. typhimurium* TA100

3. Metabolic Activation:

a. Transformation assay: None

b. Gene mutation assay:
The S9 mix was derived from rats pretreated with polychlorinated biphenyl (not further defined) and contained the following per mL: 200 or 300 µL S9 fraction, 8 µmol $MgCl_2$, 33 µmol KCl, 4 µmol NADPH, 5 µmol glucose-6-phosphate, and 100 µmol phosphate buffer. The final concentration of S9 in culture was 4 or 6%.

4. Test Cells: Transformation assay

Syrian hamster embryo cells

Properly maintained? **Yes**
Periodically checked for Mycoplasma contamination? **Not reported**
Periodically checked for karyotype stability? **Not applicable, because cells were primary cultures derived from embryos**

Media: Dulbecco's modified Eagle medium supplemented with 20% fetal bovine serum, 2 mM L-glutamine, and without antibiotics

5. Test Organisms: Gene mutation assay

S. typhimurium strains: TA98 and TA100

Properly maintained? **Yes**
Checked for appropriate genetic markers (rfa mutation, R factor)? **Not reported**

6. Test compound concentrations used:

Preliminary cytotoxicity Assay: Preliminary cytotoxicity tests were not performed in either the transformation or gene mutation assays.

a. Transformation Assays:
First test: 1, 5, 10, and 20 µg/mL
Second test: 0.1, 0.5, and 1 µg/mL
Third test: 1, 5, and 10 µg/mL

b. Gene mutation assay: 10, 25, 50, 100, and 200 µg/plate

B. TEST PERFORMANCE

Transformation assay

1. Cell treatment:

Embryos taken from Syrian golden hamsters killed on days 13 and 14 of gestation were minced and trypsinized. Inocula of 1×10^7 embryo cells were cultured in 75 cm^2 flasks at 37°C until confluent. These primary cultures were trypsinized, dispensed in lots of 5×10^6 cells in ampules, and stored in liquid nitrogen for use as target and feeder-layer cells. Prior to performing the assay, samples from eight frozen lines of embryo cells were tested for their susceptibility to transformation with 3-MC. Based on the results of this test, three lines were selected as target cells for the transformation assays, and one of these three, line 7708, was used with N,N-dimethyldodecylamine oxide.

For the assay, an ampule of cryopreserved primary cells for use as a feeder layer was thawed and plated in a 75 cm^2 flask with 20 mL of medium. On day 3 of growth, another ampule of primary cells line 7708 for use as target cells was thawed and plated. On day 4 the feeder cells (shifting from logarithmic growth to stationary phase) were irradiated with 5000 R from a linear accelerator, trypsinized, and plated at 6×10^4/60-mm dish. On day 5, 500 target cells in 2 mL of medium were added to each dish of irradiated feeder-layer cells. On day 6, an appropriate dose of the test chemical in a volume of 4 mL was added. Nine dishes were used for each dose level. Untreated, solvent, and positive controls were included. The treatment period was eight days. On day 14, the cells were fixed with absolute methanol and stained with Giemsa.

2. Colonies scored: Normal and transformed colonies were counted with a stereoscopic dissection microscope. Randomly oriented three-dimensional growth with extensive crossing-over of the cells at the periphery of the colony was considered to be the endpoint of morphological transformation. The centers of transformed colonies usually exhibit dense piling-up of cells. These cells usually have an increased ratio of nucleus to cytoplasm, are more basophilic, and are variable in size.

Gene mutation assay

1. Type of Salmonella assay: Standard plate test with preincubation step

2. Protocol: Prior to plating, inocula of the tester strains were grown overnight in nutrient broth. The test substance was dissolved in DMSO or distilled water to specified concentrations. A 0.1 mL aliquot of bacterial suspension and 0.5 mL phosphate buffer containing the test substance were preincubated at 37°C for 20 minutes prior to the addition of 2 mL melted top agar. For metabolic activation cultures, 0.5 mL of S9 mix was substituted for the phosphate buffer. The top agar was then overlaid on plates of minimal glucose agar and the plates were incubated at 37°C for 48 hours. Solvent and positive controls were included. The number of plates used at each dose level, strain, and condition was not reported. The plates were scored for revertant colonies.

3. Statistical Methods: Data were not evaluated statistically for either assay.

4. Evaluation Criteria: Criteria for a valid test and a positive response were not reported for either assay.

II. REPORTED RESULTS

The test substance was reported to have been manufactured by H_2O_2 oxidation of dimethyldodecylamine having a homologue distribution as follows: C_{10}, 0.8%, C_{12} 97.5%, C_{14}, 1.7%. It was an aqueous solution containing 29.1% active ingredient, 70.6% water, and 0.03% H_2O_2. Analytical determinations of the dose formulations were not reported for either assay.

A. Cell transformation assay: Three tests were run with 6 concentrations of N,N-dimethyldodecylamine oxide ranging from 0.1 to 20 µg/mL. Moderate toxicity, as seen by a decrease in surviving colonies, occurred at 20 µg/mL (163 vs ≥459 in the solvent control). No transformed colonies were observed in the solvent control or at any dose level in the three tests. A few (1-2) transformed colonies were observed in positive control cultures. The results of the cell transformation assay (study report Table 1, page 7) are presented as an attachment (Attachment I) to this DER.

B. Gene mutation assay: A single trial was conducted with 5 concentrations of N,N-dimethyldodecylamine oxide ranging from 10 to 200 µg/plate. The test compound was extremely toxic to both tester strains at 200 µg/plate (-S9) and slightly toxic at 100 µg/plate. No mutagenic activity was observed in the solvent control or at any dose level either with or without metabolic activation. The positive controls gave the appropriate responses. The results of the gene mutation assay

(study report Table 2, pages 8 and 9) are presented as an attachment (Attachment II) to this DER.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that, under the conditions of this study, N,N-dimethyldodecylamine oxide did not induce transformation in Syrian hamster embryo cells and was not mutagenic in *S. typhimurium* strains TA98 and TA100 with or without metabolic activation.

B. Reviewer's Discussion

The reviewers agree with the study authors' conclusion that under the conditions of this study, N,N-dimethyldodecylamine oxide neither induced cell transformation nor was mutagenic. However, in the transformation assay the positive control (3-MC) gave a very weak response; only 1-2 transformed colonies were observed out of approximately 400-500 surviving colonies per dose level and test, and 4 of the 9 reported dose levels/tests had no transformed colonies. The assay did not include any exogenous metabolic activation, which may have produced different results. In addition, the dose levels were not analyzed for actual concentrations.

In the gene mutation assay, only two *S. typhimurium* strains were used instead of the recommended four strains and only a single trial was performed. No rationale was given for dose selection, doses were not analyzed for actual concentrations, and toxicity was not reported in the presence of S9 activation at the highest dose level of 200 µg/plate , which is well below the recommended limit dose of 5000 µg/plate. In addition, it could not be determined whether DMSO or water served as vehicle for the test compound. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. This study, which reports the results of a cell transformation assay and a microbial gene mutation assay, is unacceptable and cannot be upgraded.

IV. STUDY DEFICIENCIES

The following deficiencies were noted for this study: (i) the positive control in the transformation assay produced a weak response, (ii) the transformation assay did not include an exogenous metabolic activation system, (iii) only two instead of

the recommended four tester strains of *S. typhimurium* were used in the microbial gene mutation assay, (iv) the vehicle for the test compound in the gene mutation assay was not specified, and, (v) the highest dose level in the gene mutation assay was well below the recommended limit dose and in the presence of S9 activation, toxicity was not reported at any dose level. In addition, the rationale for dose selection was not reported for either assay and doses were not analyzed for actual concentrations; the lot number of the test compound was not provided; the number of plates used at each dose level in the gene mutation assay was not reported and a confirmatory test was not run; and statistical analysis was not performed and evaluation criteria were not reported for either assay.

ATTACHMENT 1

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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Table 1. *Transformation of cryopreserved hamster embryo cells by ten surfactants and two other compounds*

Compound	Cell culture	No. of transformed colonies/No. of surviving colonies															
		0·2% DMSO‖	3MC‖ (μg/ml)			Dose of test material (μg/ml)											
			0·1	0·5	1·0	0‖	0·01	0·05	0·1	0·5	1	5	10	20	50	100	300
Anionic surfactants																	
Linear alkylbenzene sulphonate	7708	0/607	1/512	0/438	0/430	0/601	—	—	—	—	—	0/496	0/399	0/371	0/241	—	—
	7708	0/603	0/538	1/527	0/500	0/620	—	—	—	0/617	0/608	0/595	0/544	—	—	—	—
Sodium alkylpoly-(oxyethylene) sulphate	7708	0/607	1/512	0/438	0/430	0/601	—	—	—	—	—	0/508	0/512	0/500	0/408	—	—
Cationic surfactants																	
Cetyltrimethylammonium chloride	7708	0/470†	1/504	0/520	0/490	0/545	—	—	0/588	—	0/413	0/9	—	—	—	—	—
	7708	0/546	0/441	0/410	1/422	0/552	—	0/515	0/499	0/438	—	—	—	—	—	—	—
Dicetyldimethylammonium chloride	7708	0/603	0/538	1/527	0/500	0/620	—	0/582	0/577	0/574	0/566	—	—	—	—	—	—
Cetyldimethylbenzylammonium chloride	7708	0/603	0/538	1/527	0/500	0/620	0/583	0/606	0/576	0/496	—	—	—	—	—	—	—
Nonionic surfactants																	
N,N-Bis(2-hydroxyethyl)lauramide	7708	0/607	1/512	0/438	0/430	0/601	—	—	0/535	0/512	0/493‖	0/483	0/472	—	—	—	—
	7708	0/604	0/569	2/543	0/519	0/609	—	—	0/620	—	0/598	—	0/614	—	—	—	—
	7708	0/546	0/441	0/410	1/422	0/552	—	—	0/528	—	0/537	—	0/553	—	—	—	—
Polyoxyethylene sorbitan monostearate	7904	0/230	1/204	0/202	0/223	0/260	—	—	—	—	0/270	—	0/268	—	0/202	0/200	0/0
	7904	0/329	1/300	3/277	1/252	0/341	—	—	—	—	—	—	0/310	—	0/285	0/220	—
Sorbitan monostearate	7904	0/230	1/204	0/202	0/223	0/260	—	—	—	—	0/237	—	0/229	—	0/198	0/164	0/63
	7904	0/329	1/300	3/277	1/252	0/341	—	—	—	—	—	—	0/310	—	0/258	0/245	—
Amphoteric surfactants																	
N,N-Dimethyldodecylamine oxide (C_{12})	7708	0/459‡	0/395†	1/414	1/378	0/520†	—	—	—	—	0/366	0/357	0/357	0/163	—	—	—
	7708	0/592	1/541	2/467†	0/520	0/581	—	—	0/565	0/541	0/552	—	—	—	—	—	—
	7708	0/579	0/409†	2/441	0/432	0/623	—	—	—	—	0/548	0/564	0/502	—	—	—	—
N,N-Dimethyltetradecylamine oxide (C_{14})	7708	0/592	1/541	2/467	0/520	0/581	—	—	0/568	0/549	0/558	—	—	—	—	—	—
	7708	0/579	0/409†	2/441	0/432	0/623	—	—	—	—	0/568	0/490	0/192	—	—	—	—
	7708	0/604	0/569	2/543	0/519	0/609	—	—	—	0/594	0/581	0/579	—	—	—	—	—
Other compounds																	
N-Nitrosomethyl-*n*-dodecylamine	7708	0/644	0/585	1/550	1/524	0/652	—	—	—	—	0/681	—	0/670	—	—	0/0	—
Dimethylglyoxime	7602	0/376	2/293	2/227	1/203	0/368	—	—	0/316	—	—	0/294	2/305	—	—	—	—
	7602	0/427	2/263	0/216	0/226	0/462	—	—	2/461	—	2/458	—	3/461	—	—	—	—

DMSO = Dimethylsulphoxide MC = 3-Methylcholanthrene

*In all cases nine dishes each seeded with *c.*500 target cells were used except where marked with daggers (†eight dishes were used; ‡seven dishes were used).

‖Cells treated with dimethylsulphoxide provided the solvent control, 3-methylcholanthrene, the positive control and culture medium alone, the tissue culture control.

ATTACHMENT 2

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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Table 2. *Mutagenicity of ten surfactants and two other compounds for* S. typhimurium *strains TA100 and TA98*

Compound	Dose (μg/plate)	No. of His⁺ revertants/plate with or without S-9 mix using strain			
		TA100		TA98	
		(−) S-9	(+) S-9	(−) S-9	(+) S-9
Controls					
H_2O	—	145	137	19	32
DMSO	—	151	141	23	28
4-Nitroquinoline 1-oxide	0·5	1588	148	167	36
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine	2	4152	128	27	30
Benzo[*a*]pyrene	5	178	928	35	614
2-Acetylaminofluorene	50	128	1080	18	2292
N-Nitrosodimethylamine	3000	143	3154	20	34
Linear alkylbenzene sulphonate	10	152	101	38	26
	25	174	105	23	32
	50	190	103	23	24
	100	179	126	22	23
	200	104	118	17	28
Soduim alkylpoly(oxyethylene) sulphate	10	136	130	23	32
	50	130	132	20	31
	100	114	134	12	32
	200	118	134	22	30
	1000	10	117	13	21
Cetyltrimethylammonium chloride	0·05	122	105	12	18
	0·1	121	110	12	30
	0·5	127	123	19	38
	1	105	103	14	22
	5	0*	127	0*	24
	10	0*	133	0*	25
Dicetyldimethylammonium chloride	1	114	129	19	23
	10	148	114	23	30
	50	142	122	15	35
	100	133	141	12	40
	200	129	143	18	30
	400	130	125	15	32
Cetyldimethylbenzylammonium chloride	0·01	123	139	12	29
	0·05	133	108	13	22
	0·1	105	120	10	40
	0·5	100	115	17	18
	1	96	129	15	26
	5	0*	132	0*	28
	10	0*	91	0*	18
N,N-Bis(2-hydroxyethyl)lauramide	10	120	118	23	31
	50	110	105	18	29
	100	130	126	19	27
	200	37	113	11	30
	1000	0	91	0	18
Polyoxyethylene sorbitan monostearate	10	121	111	18	31
	100	134	127	23	29
	200	157	133	33	34
	1000	148	141	22	33
	2000	115	136	24	33
Sorbitan monostearate	10	137	133	17	25
	100	131	123	14	36
	200	150	134	22	32
	1000	133	142	16	30
	2000	130	157	17	30
N,N-Dimethyldodecylamine oxide	10	134	141	20	31
	25	131	137	25	35
	50	140	116	25	26
	100	116	140	12	28
	200	0*	145	0*	39
N,N-Dimethyltetradecylamine oxide	10	123	155	22	29
	25	113	140	23	28
	50	112	120	27	30
	100	60	159	16	36
	200	11	140	14	27

(*Continued on next page*)

Table 2. (*Continued*)

Compound	Dose (μg/plate)	No. of His⁺ revertants/plate with or without S-9 mix using strain			
		TA100		TA98	
		(−) S-9	(+) S-9	(−) S-9	(+) S-9
N-Nitrosomethyl-*n*-dodecylamine	50	123	158	21	20
	100	112	206	23	26
	200	62	360	11	33
	300	63	432	13	27
	500	40	314	5	21
	1000	NT	180	NT	28
	3000	NT	142	NT	20
	5000*	NT	148	NT	14
Dimethylglyoxime	10	118	174	16	31
	50	133	158	23	27
	100	157	143	20	31
	500	135	141	12	36
	1000	132	133	16	28
	2000	152	149	20	33

NT = Not tested DMSO = Dimethylsulphoxide

*The compound was particularly toxic to the bacteria at these doses.

similar positive response was noted for *N*-nitrosodimethylamine with strain TA100.

None of the ten surfactants assayed showed any mutagenic activity against TA100 and TA98 either with or without activation. Of the two compounds, *N*-nitrosomethyl-*n*-dodecylamine showed significant but low mutagenic activity using TA100 after metabolic activation. Dimethylglyoxime was not mutagenic towards either strain.

DISCUSSION

There are now various short-term screening tests for the identification of environmental carcinogens (Bridges, 1976; Montesano *et al.* 1976; Stoltz *et al.* 1974). Of these, it has been indicated that the *Salmonella*/microsome mutagenicity test and an *in vitro* cell transformation assay are both reasonably efficient at predicting carcinogenicity in animals. Although none of these tests can replace long-term tests in animals, they are of great value in establishing priorities for long-term testing.

Two promising cell systems have been developed as *in vitro* models for the study of chemical carcinogenesis: one system utilizes heterogeneous primary or secondary cultures of hamster embryo cells (Berwald & Sachs, 1963 & 1965; DiPaolo, Donovan & Nelson, 1969; DiPaolo, Nelson & Donovan, 1969; DiPaolo *et al.* 1971; Huberman & Sachs, 1966; Kuroki & Sato, 1968); while the other uses clonable long-term lines of mouse fibroblasts (Chen & Heidelberger, 1969a,b,c; DiPaolo, Takano & Popescu, 1972; Kakunaga, 1973; Marquardt, Sondergren, Simp & Grover, 1974; Mondel & Heidelberger, 1970; Reznikoff, Bertram, Brankow & Heidelberger, 1973). In this study, we used cryopreserved primary cultures of Syrian golden hamster embryo cells as the source of target and feeder layer cells for *in vitro* carcinogenesis bioassay (Pienta *et al.* 1977). It was demonstrated that target cells freshly prepared from pools of 13-day-old hamster embryos vary in their susceptibility to a known carcinogen, 3-methylcholanthrene (Fig. 1). Pienta *et al.* (1977) found that about one-half of the cells from eleven secondary cultures were not transformed by 3-methylcholanthrene at any of the doses tested, a further indication that not all samples of cells are susceptible to transformation. We found that all the samples of freshly prepared early passage hamster embryo cells showed responses, although they varied among different pools. In the present work we used three different cultures giving the overall responses in a previous test to minimize non-responsiveness with cells.

Of the ten surfactants tested, three surfactants, namely LAS (Bornmann & Loeser, 1963; Buehler, Newman & King, 1971) sodium alkylpoly(oxyethylene) sulphate (Tusing, Paynter, Opdyke & Synder, 1962) and CDBAC (Alfredson, Stiefel, Thorp, Barten & Gray, 1951; Fitzhugh & Nelson, 1948), have been found to be non-carcinogenic in animal experiments. Although CTAC has not been tested for carcinogenic effects, its analogue, cetyltrimethylammonium bromide (Isomaa, Reuter & Djupsund, 1976) had none in rats. These four surfactants did not induce *in vitro* transformation of hamster embryo cells neither were they mutagenic. The two anionic surfactants, Tween 60 and Span 60, gave negative results for transformation and mutagenicity. It was reported that these surfactants were not carcinogenic when they were fed to mice, rats, hamsters or dogs (Brush, McCoy, Rosenthal, Stauber & Allison, 1957; Fitzhugh, Bourke, Nelson & Frawley, 1959; Oser & Oser, 1957). However, it has been reported that Tween 60 is a weak promoting agent (Setälä, 1956; Setälä, Setälä & Holsti, 1954) and a weak carcinogen (Della Porta, Shubik, Dammert & Terracini, 1960; Shubik, Della Porta & Spencer, 1959) after topical application to mouse skin, and also produces local sarcomas after sc